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Distribution and taxonomy of two closely related *Nychiodes* species in southern Italy (Lepidoptera, Geometridae, Ennominae)

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Abstract. We investigated the distribution and species circumscription of *Nychiodes obscuraria* Villers (1789) and *Nychiodes ragusaria* Millière (1884). The morphology of male and female genitalia of Calabrian populations (southern Italy) revealed them to belong to *N. ragusaria* while specimens from Basilicata, Molise and the rest of Italy turned out to be *N. obscuraria*. Although the DNA barcodes showed a divergence of 1.55% between typical *N. obscuraria* and *N. ragusaria*, 9 out of 28 Calabrian specimens of *N. ragusaria* shared the *N. obscuraria* haplotype, and sometimes both haplotypes were found at the same site. Genetic introgression appears as the most probable scenario to explain the observed pattern. A correct identification for the investigated species pair should be based on morphology instead of DNA barcode analysis because of the observed mitonuclear discordance at mountain sites. Further studies are needed to clarify the occurrence of this discordance at high elevations only.

Introduction

The genus *Nychiodes* comprises 27 species (Rajaei et al. 2022), currently assigned to the Boarmiini, a tribe of geometrid moths including more than 5,000 species (Müller et al. 2019; Murillo-Ramos et al. 2019). The genus is distributed in the Palaearctic region from Western Europe and North Africa to Iran, Afghanistan, and Pakistan. *Nychiodes* species are large, robust moths, forming a diverse group based on external morphology, without well-defined differential characters (Lederer 1853; Müller et al. 2019). Species are large-sized, with females being slightly larger than males. Wings are light brown to beige, dark grey, or with shades of brown, wing tip is slightly darker. *Nychiodes* species are mainly characterized by prominent ante- and postmedial lines on the forewing (Müller et al. 2019). Discal spots are usually present on all wings, of medium size. Antennae are dorsally covered with a mixture of yellowish and brown scales, bipectinate in both sexes, branches long in males, and short in females. The proboscis is absent. Despite the conspicuousness of this genus, its morphological variability makes it difficult to identify and classify species. Therefore, the study of genitalia structures is essential for a clear diagnosis of the species.

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Based on the morphology of the genitalia, the genus *Nychiodes* has been divided into three distinct species-groups: *obscuraria* species-group, *waltheri* species-group, and *amygdalaria* species-group (Wehrli 1929a, b; Müller et al. 2019). However, this grouping should not be considered as a phylogenetic concept until confirmed by a thorough molecular phylogenetic study. The *obscuraria* species-group can be diagnosed on the basis of the following characteristics according to Müller et al. (2019): In male genitalia, the costa of the valva is sclerotized to the subapical part, humped medially; the apex of the upper ampulla is covered with setae; the lower ampulla is not setose, pointed; the process of the sacculus is pointed; the tip of the aedeagus bears an acute triangular extension. In the female genitalia, the ductus bursae is long and membranous (equal to or longer than corpus bursae); lamella postvaginalis is strongly extended laterally and sclerotized. The following European species are included in this group: *N. obscuraria* Villers (1789), *N. ragusaria* Millière (1884), *N. andalusiaria* Millière (1865), *N. notarioi* Hermosa (2005), *N. mauretanica* Wehrli (1929c), *N. hispanica* Wehrli (1929c). The work focuses on two species of the genus *Nychiodes*, *N. obscuraria* and *N. ragusaria*. In Italy, the presence of *N. obscuraria* is confirmed in Valle d'Aosta, Piedmont, Lombardy, Trentino, South Tyrol, Veneto, Liguria, Emilia, Romagna, Tuscany, Umbria, Marche, Abruzzo, Molise, Puglia, Basilicata (Parenzan and Porcelli 2007). In southern Italy there are individuals of different size and with more or less marked wing pattern. The species is widespread and frequent, especially in hilly and mountainous areas, flying from early June to late July (Parenzan 1994). *N. ragusaria*, on the other hand, was previously considered an endemism of Sicily but Parenzan (1994) reported *N. ragusaria* as a new for the peninsula, occurring in xerothermic sites, both coastal and mountainous and being common from late June to mid-September. *N. ragusaria* has been confirmed in several locations in Calabria. In Calabria we found some individuals with contrasting mitochondrial DNA (mtDNA) and morphology. When two species externally similar exhibit significant variation in colouration, mtDNA analysis is often used for correct identification. However, in this particular case, mtDNA alone was insufficient. Dissection of the genitalia revealed discrepancies between the mitochondrial and morphological characteristics of some individuals collected from certain localities.

The aim of the work is to analyze a series of samples collected in different parts of Italy, in particular: (1) to investigate the potential presence of *N. obscuraria* in Calabria (southern Italy), through DNA barcoding and morphology of genitalia; (2) to refine the boundaries between both species as potential hybridization zones.

Material and methods

One hundred and sixty-eight specimens were analyzed, used for comparison of internal characters combined with DNA data and distribution patterns. Standard protocols (e.g., Ivanova et al. 2006) were used for DNA extraction and amplification of the 'barcode' fragment (658 base pairs of the 5' terminus) of mitochondrial Cytochrome C Oxidase subunit I (COI). DNA extraction, PCR and sequencing were performed at the Canadian Centre for DNA Barcoding (CCDB, Guelph). All samples used for DNA analysis are presented in Table 1 along with their label data and sample ID. All sequences and metadata are available on BOLD in the public dataset DS-NYCHSITAL. MEGA X software (Kumar et al. 2018) was used to reconstruct maximum likelihood (ML) tree (Fig. 6) (using the K2P model: Kimura 1980). Genetic p-distances were calculated using the barcode gap analysis function on BOLD. The illustration of distributional data was created using Google Earth

Table 1. List of specimens used for DNA analysis.

Taxon identification	Collection data	Sex	COI bp	BOLD sample ID
N. obscuraria	Italy, Trentino-Alto Adige, Vinschgau, Staben, 700 m, 18 vii 1984, leg. E. Loser	♀	601	BMB Lep 00031
N. obscuraria	Italy, Piemonte, Val di Susa, Chiomonte	♂	658	LEP-SS-01052
N. obscuraria	Italy, Emilia-Romagna, Forli, Castrocaro Terme, 150 m, 1 vii 2008, leg. G. Govi	♂	658	GF Lep 0076
N. obscuraria	Italy, Basilicata, Lucania, Monte Caperino, Italy, Pietrapertosa, 1400 m, 27 vii 1975, leg. Parenzan	♂	0	BC ZSM Lep 15073
N. obscuraria	Italy, S. p. Mad. Di Sirino, Lagonegro, Basilicata, 1065 m, 27 vii, 2020, 40.119°N, 15.802°E	♀	658	BC ZSM Lep 111806
N. ragusaria	Italy, Purgatorio SB Ullano, Calabria (CS) 845 m 28 vi 2016, 39.3983°N, 16.1046°E, leg. Scalercio & Infusino	♂	658	LEP-SS-00773
N. ragusaria	Italy, Sciortaglie-Alessandria d.C. (CS) 1246 m 19 vii 2017, 39.9313°N, 16.3508°E, Leg. Scalercio & Infusino	♂	658	LEP-SS-00771
N. ragusaria	Italy, Dif. Privitera-Alessandria d.C. (CS) 1314 m, 19 vii 2017, 39.9269°N, 16.3563°E, leg. Scalercio & Infusino	♀	658	LEP-SS-00770
N. ragusaria	Italy, II Palmento, S.S. Bruno (VV) 840 m, 13 vii 2015, 38.5625°N, 16.3140°E, leg. Scalercio & Infusino	♂	658	LEP-SS-01058
N. ragusaria	Italy, Sila, Vivaio Sbanditi (CS) 1350 m, 24 vii 2014, 39.2320°N, 16.3608°E, leg. S. Scalercio,	♂	0	LEP-SS-01057
N. ragusaria	Italy, Tappaiolo-Alessandria d.C. (CS) 1253 m, 19 vii 2017, 39.9358°N, 16°3471°E, leg. Scalercio e Infusino	♀	658	LEP-SS-00774
N. ragusaria	Italy, Colle Macchie, Pedace (CS) 1440 m, 17 vii 2015, 39.2589°N, 16.5272°E, leg. Scalercio & Infusino	♂	658	LEP-SS-00775
N. ragusaria	Italy, Fago del Soldato, Spezzano Sila (CS) 1402 m, 6 vii 2018, 39.356922°N, 16.407934°E, leg. S. Scalercio,	♂	658	LEP-SS-01053
N. ragusaria	Italy, Fiego di San Fili (CS) 740 m, 22 vii 2015, 39.3288°N, 16.1286°E, leg. Scalercio & Infusino	♂	658	LEP-SS-01054
N. ragusaria	Italy, C. da S. Cenere, Soveria-Simeri (CZ) 86 m, 25 vi 2020, 38.9259°N, 16.6729°E, leg. S. Scalercio	♂	658	LEP-SS-01066
N. ragusaria	Italy, C. da S. Cenere, Soveria-Simeri (CZ) 86 m, 01 x 2019, 38.9259°N, 16, 6729°E, leg. S. Scalercio,	♂	658	LEP-SS-01056
N. ragusaria	Italy, C. da Licari, Marcellinara (CZ) 195 m, 10 vi 2019, 38.9180°N, 16.4961°E, leg. S. Scalercio,	♂	658	LEP-SS-01065
N. ragusaria	Italy, C.da Licari, Marcellinara (CZ) 210 m, 05 x 2018, 38.9189°N, 16.4971°E, leg S. Scalercio	♂	658	LEP-SS-01063
N. ragusaria	Italy, Ariabrutta, Sellia (CZ) 470 m, 14 v 2020, 38.98865°N, 16.62183°E, leg. S. Scalercio	♂	658	LEP-SS01060
N. ragusaria	Italy, C.da Licari, Marcellinara (CZ) 195 m, 08 viii 2018, 38.9180°N, 16.4961°E, leg. S. Scalercio	♂	645	LEP-SS-01064
N. ragusaria	Italy, Vitrò, Sellia (CZ) 161 m, 24 vii 2019, 38.95856°N, 16.61923°E, leg. S. Scalercio	♂	658	LEP-SS-01059
N. ragusaria	Italy, Maierato (VV) Scuotrapiti-Lago Angitola, 44 m, 21 vi 2002, leg. Scalercio, Infusino & Tuscano	♂	0	LEP-SS-01055
N. ragusaria	Italy, Simeri-Crichi (CZ) 425 m, 05 ix 2019, 38.9567°N, 16.6489°E, leg. S. Scalercio	♂	658	LEP-SS-01055
N. ragusaria	Italy, Vaccaro, Sellia (CZ) 391 m, 05 ix 2019, 38.96641°N, 16.62486°E, leg. S. Scalercio	♀	658	LEP-SS-01062
N. ragusaria	Italy, Calabria centr. Sila, Lago Cecita, Longobucco 1170 m, 13 viii 2013	♂	658	BC ZSM Lep 75110
N. ragusaria	Itlay, Monte Pollino, Terranova, 900 m, 3 vi 1981	♂	0	BC ZSM Lep 14346
N. ragusaria	Italy, Bosco di Malabotta, Sicily, 1320 m, 8 viii 2007, 37.967°N, 15.067°E	♀	658	BC ZSM Lep 12514
N. ragusaria	Italy, Coccorina, Tropea, Calabria, 200 m, 12 vi 2008, 38.6458°N, 15.8869°E	♂	658	BC ZSM Lep 24336
N. ragusaria	Italy, Coccorina, Tropea, Calabria, 200 m, 12 vi 2008, 38.6458°N, 15.8869°E	♂	658	BC ZSM Lep 24335
N. ragusaria	Italy, Coccorina, Tropea, Calabria, 200 m, 12 vi 2008, 38.6458°N, 15.8869°E	♂	658	BC ZSM Lep 24334

Pro (ver. 7.3.6.9345 for Mac). Male and female genitalia were prepared according to standard procedures (e.g., Robinson 1976), embedded in Euparal and photographed with an Olympus E-M1 camera, stacking the different focus layers with Helicon Focus 6 software.

The species studied were from the following collections.

CREA-FL Collection of the Research Centre for Forestry and Wood (Rende, Italy).
SNSB-ZSM Bavarian State Collection of Zoology (Munich, Germany).
CAS Collection of Andrea Sciarretta (Molise, Italy).

Results

Nychiodes ragusaria Millière (1884)

Nychiodes lividaria var. *ragusaria* Millière (1884): Naturalista sicil. 3 (7), (Italy: Sicily, Castelbuono). Neotype ♂ (ZFMK, designated by Fazekas (1997).
Nychiodes bellieraria Ragusa (1884): Naturalista sicil. 3 (12), (Italy: Sicily, Castelbuono). Junior synonym of *N. ragusaria* proposed by Fazekas (1997), confirmed by Flamigni et al. (2007).

Material examined. Italy: Calabria: ♂, Purgatorio, S.B. Ullano (CS) 845 m 28 vi 2016, 39.3983°N, 16.1046°E, leg. Scalercio & Infusino, Lep-SS-00773 (CREA-FL); ♂, Sciortaglie-Alessandria d.C. (CS) 1.246 m 19 vii 2017, 39.9313°N, 16.3508°E, leg. Scalercio & Infusino, LEP-SS-00771 (CREA-FL); ♀, Dif. Privitera-Alessandria d.C. (CS) 1314 m, 19 vii 2017, 39.9269°N, 16.3563°E, leg. Scalercio & Infusino, LEP-SS-00770 (CREA-FL); ♂, II Palmento, S.S. Bruno (VV) 840 m, 13 vii 2015, 38.5625°N, 16.3140°E, leg. Scalercio & Infusino, LEP-SS-01058 (CREA-FL); ♂, Sila, Vivaio Sbanditi (CS) 1350 m, 24 vii 2014, 39.2320°N, 16.3608°E, leg. S. Scalercio, LEP-SS-01057 (CREA-FL); ♀, Tappaiolo-Alessandria d.C. (CS) 1253 m, 19 vii 2017, 39.9358°N, 16.3471°E, leg. Scalercio e Infusino, LEP-SS-00774 (CREA-FL); ♂, Colle Macchie, Pedace (CS) 1440 m, 17 vii 2015, 39.2589°N, 16.5272°E, leg. Scalercio & Infusino, LEP-SS-00775 (CREA-FL); ♂, Fago del Soldato, Spezzano Sila (CS) 1402 m, 6 vii 2018, 39.356922°N, 16.407934°E, leg. S. Scalercio, LEP-SS-01053 (CREA-FL); ♂, Fiego di San Fili (CS) 740 m, 22 vii 2015, 39.3288°N, 16.1286°E, leg. Scalercio & Infusino, LEP-SS-01054 (CREA-FL); ♂, C. da S. Cenere, Soveria-Simeri (CZ) 86 m, 25 vi 2020, 38.9259°N, 16.6729°E, leg. S. Scalercio, LEP-SS-01066 (CREA-FL); ♂, C. da S. Cenere, Soveria-Simeri (CZ) 86 m, 01 x 2019, 38.9259°N, 16.6729°E, leg. S. Scalercio, LEP-SS-01056 (CREA-FL); ♂, Cda Licari, Marcellinara (CZ) 195 m, 10 vi 2019, 38.9180°N, 16.4961°E, leg. S. Scalercio, LEP-SS-01065 (CREA-FL); ♂, C. da Licari, Marcellinara (CZ) 210 m, 05 x 2018, 38.9189°N, 16.4971°E, leg S. Scalercio, LEP-SS-01063 (CREA-FL); ♂, Ariabrutta, Sellia (CZ) 470 m, 14 v 2020, 38.98865°N, 16.62183°E, leg. S. Scalercio, LEP-SS-01060 (CREA-FL); ♂, C. da Licari, Marcellinara (CZ) 195 m, 08 viii 2018, 38.9180°N, 16.4961°E, leg. S. Scalercio, LEP-SS-01064 (CREA-FL); ♂, Vitrò, Sellia (CZ) 161 m, 24 vii 2019, 38.95856°N, 16.61923°E, leg. S. Scalercio, LEP-SS-01059 (CREA-FL); ♂, Maierato (VV) Scuotrapiti-Lago Angitola, 44 m, 21 vi 2002, leg. Scalercio, Infusino & Tuscano, LEP-SS-01055 (CREA-FL); ♂, Scuotrapiti, L. Angitola, 50 m, 27 vi 2001, leg. Scalercio & Infusino (SNSB-ZSM); ♂, Simeri-Crichi (CZ) 425 m, 05 ix 2019, 38.9567°N, 16.6489°E, leg. S. Scalercio, LEP-SS-01061 (CREA-FL); ♀, Vaccaro, Sellia (CZ) 391 m, 05 ix 2019, 38.96641°N, 16.62486°E, leg. S. Scalercio, LEP-SS-01062 (CREA-FL); ♂, Monte Cocuzzo (CS) 1110 m, 20 vii 1997, leg. S. Scalercio (SNSB-ZSM); ♂, Ianni-Pirillo, Monte Cocuzzo 1110 m, 19 vii 2001, leg. Scalercio & Sposato (SNSB-ZSM); ♂, Moschereto, Civita, 850 m 29 vi 1996, leg. S. Scalercio (SNSB-ZSM); ♂, Fiumara Trionto (CS), 90 m, 28 viii 2000, leg Scalercio & Infusino (SNSB-ZSM); ♂, Fiumara Trionto (CS), 90 m, 8 vii 1999, leg Scalercio, Infusino & Vuono (SNSB-ZSM); ♂, Loc. Donnici, Fosso Cucolo, 550 m, 13 vii 1996, leg. S. Scalercio (SNSB-ZSM); ♂, Loc. Donnici, Fosso Cucolo, 550 m, 6 vii 1996, leg. S. Scalercio (SNSB-ZSM); ♂, Loc. Donnici, Fosso Cucolo, 550 m, 17 vii 1996, leg. S. Scalercio (SNSB-ZSM); ♂,

Sila, Lago Cecita, Longobucco, 1170 m, 13 vii 2013, 39.3865°N, 16.5520°E, leg. A. Hausmann, Lep 75110 (SNSB-ZSM); ♂, Sersale (CZ), 500 m, 22–29 vi 1985 (SNSB-ZSM); ♂, Sersale (CZ), 500 m, 14 ix 1985 (SNSB-ZSM); ♂, Terranova, 900 m, 3 vi 1981, Lep 14346 (SNSB-ZSM); ♂, Coccorina, Tropea (VV), 200 m, 12 VI 2008, 38.6458°N, 15.8869°E; Lep 224336, leg. Schneider, Leipnitz (SNSB-ZSM); ♂, Coccorina, Tropea (VV), 200 m, 12 vi 2008, 38.6458°N, 15.8869°E; Lep 224335, leg. Schneider, Leipnitz (SNSB-ZSM); ♂, Coccorina, Tropea (VV), 200 m, 12 vi 2008, 38.6458°N, 15.8869°E; Lep 224334, leg. Schneider, Leipnitz (SNSB-ZSM); **Sicily:** ♀, Bosco di Malabotta (ME) 1320 m, 8 viii 2007, 37.967°N, 15.067°E, leg. M. Infusino, Lep 12514 (SNSB-ZSM); 3♀, 6♂, Madonie (SNSB-ZSM); 11♀, 24♂, Mistretta (SNSB-ZSM).

Description of external characters and diagnosis. (Fig. 1A) Wingspan 32–48 mm. Wings light to dark brown, with reddish-brown scales on costal area, faint blackish spots of transversal lines at costa. Forewing antemedial line curved outwards near costa, partly visible: medial line often well developed towards inner margin (completely absent in *N. obscuraria*); postmedial line angled outwards on M1 vein, slightly curved inwards, ending in the middle of inner margin. Medial line opaque, barely visible, postmedial line slightly curved on M2 vein (straight or slightly curved in *N. obscuraria*). Tiny blackish discal spots barely visible on hindwings. Terminal line of all wings blackish, narrow, discontinuous, stronger between vein endings with a yellow shadow. Fringe brown to slightly darker grey on vein endings. Underside of wings dull, beige to dark brown, outer parts slightly darker (Müller et al. 2019).

Male genitalia. (Fig. 3A) Valva extended, its dorsal part triangular, fused apically with digitiform extension (important diagnostic character against all other species of *obscuraria* group), covered with fine bristles. Only basal part of the costa of the valva sclerotized, basally gibbous. Upper ampulla clubbed, apically covered with tiny spines. Process of the lower ampulla, apically blunt, strongly sclerotized, not septate, margin of valva between ampulla inferior and sacculus strongly concave. Aedeagus large, curved in the centre. Cornutus needle-like slightly shorter than the aedeagus, slightly curved in the centre (Müller et al. 2019).

Female genitalia. (Fig. 2A) Lamella postvaginalis apically divided and bilobed, dorsolaterally larger than that of *N. obscuraria*. Ductus bursae posteriorly sclerotized, longitudinally striate, towards corpus bursae membranous. The latter large, membranous. Signum stellate.

Variation. Wing colour quite variable, as well as markings. Sometimes wing markings completely faded (Müller et al. 2019).

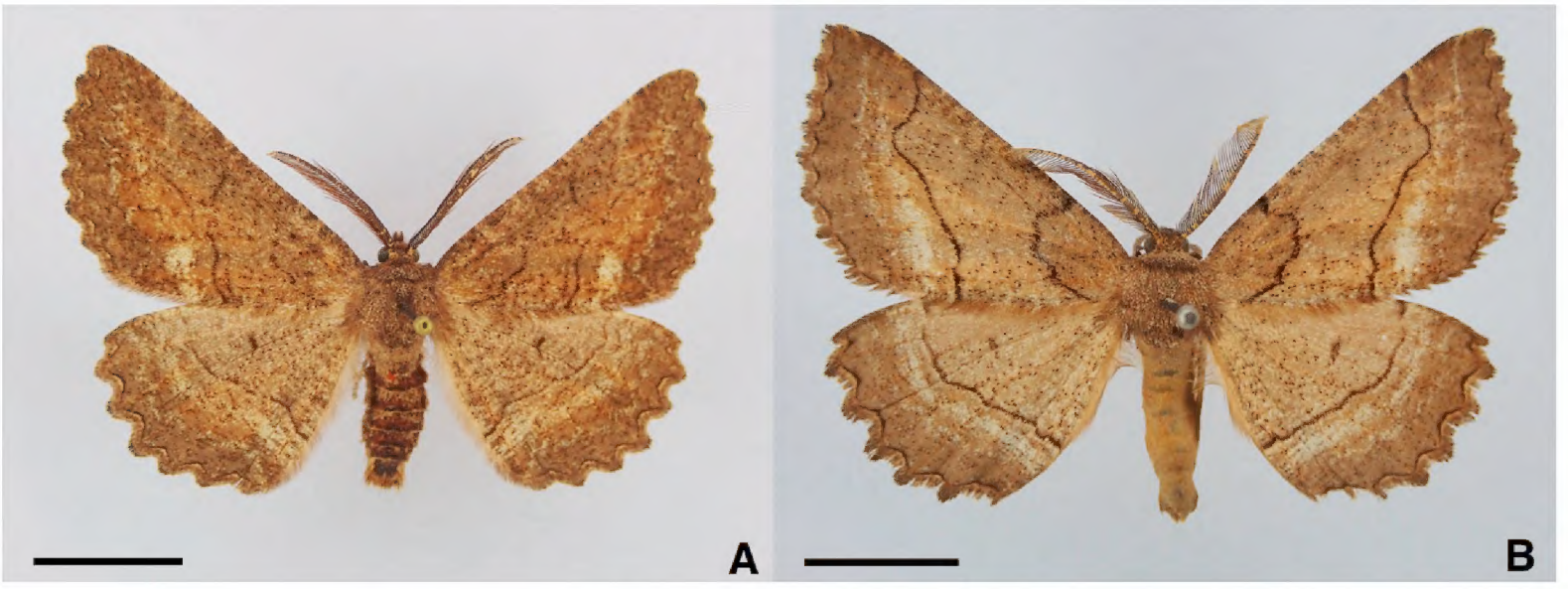

Figure 1. A. *N. ragusaria*, adult male. Italy, Sicily (ZSM); **B.** *N. obscuraria*, adult male. France, Hautes Alpes (ZSM). Scale bars: 1 cm.

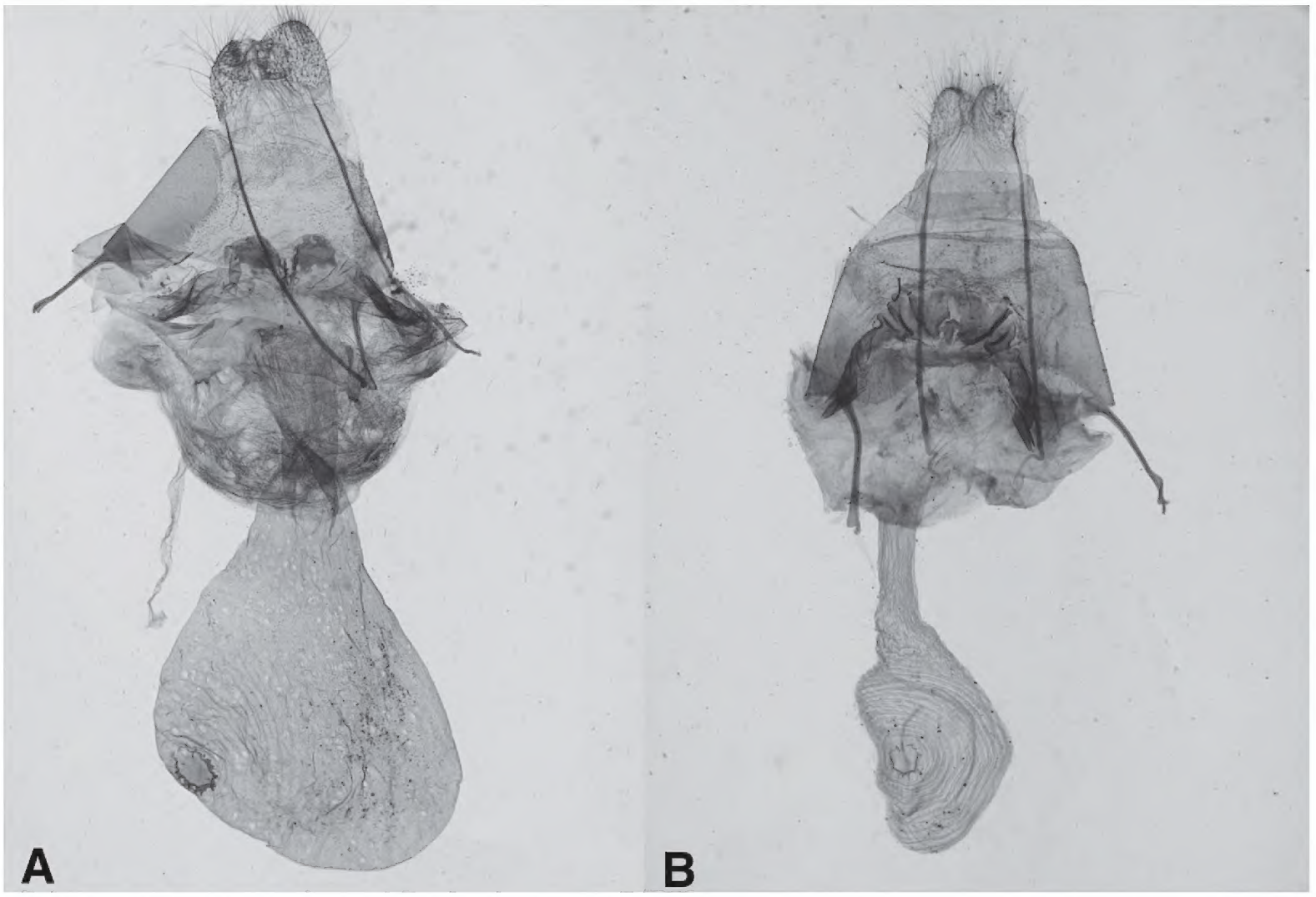

Figure 2. Female genitalia. **A.** *Nychiodes ragusaria*. Calabria, Dif. Privitera, Alessandria del Carretto (CREA-FL); **B.** *N. obscuraria*. Central Italy, Monti Sabini (ZSM).

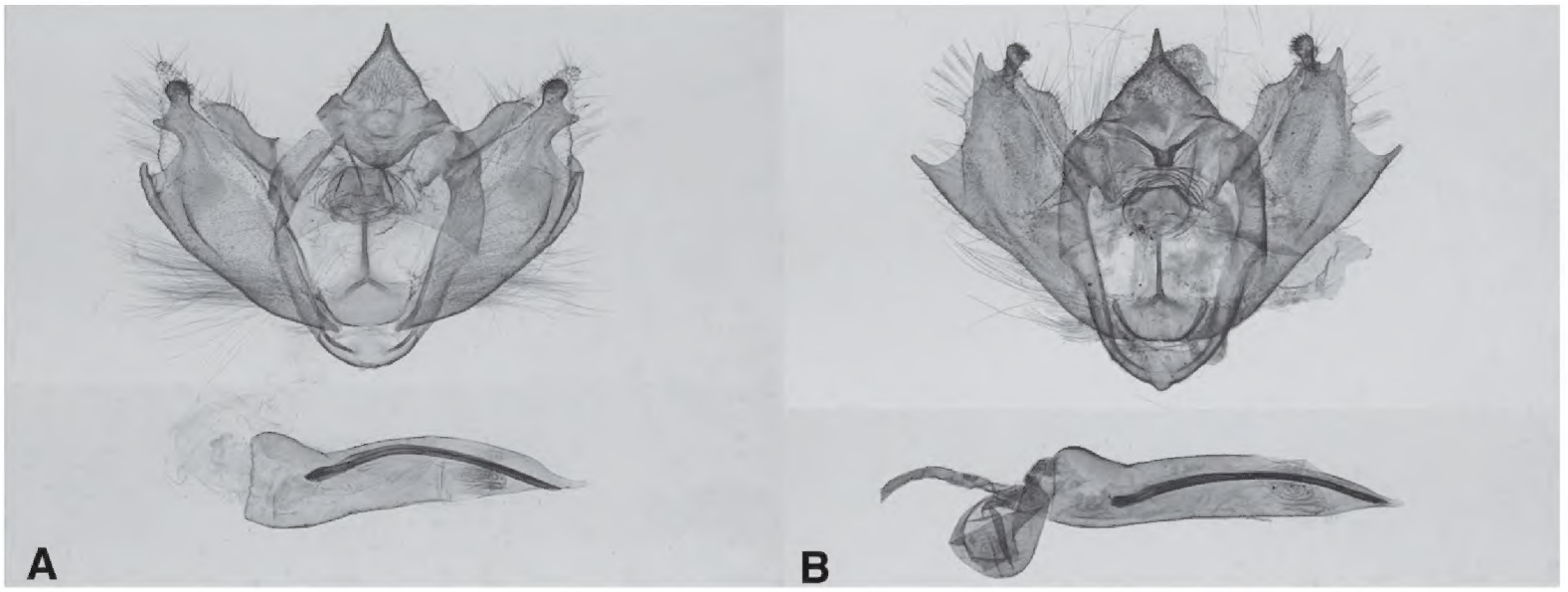

Figure 3. Male genitalia. **A.** *Nychiodes ragusaria*, Calabria, Sersale (ZSM); **B.** *N. obscuraria*. Basilicata, Pietrapertosa (ZSM).

Genetic data. Two BINs in Calabria: BOLD: AAE5581 (n = 16, sharing the BIN with typical populations from Sicily) and BOLD: ACE5020 (*N. ragusaria*: n = 9 from Calabria), the latter BIN-sharing with *N. obscuraria* from the rest of Italy and from France (*N. obscuraria*: n = 7) and exact haplotype-sharing with one specimen of *N. obscuraria* from southernmost Basilicata region. P-distance between the two South Italian haplotypes of *N. ragusaria* 1.55% in BOLD DNA barcode gap analysis. The identity of all barcoded specimens was verified by dissection of genitalia.

Nychiodes obscuraria Villers (1789)

Phalaena (*Geometra*) *obscuraria* Villers 1789): Linn. Ent 2: 325 (southern France). Neotype ♂ (ZFMK, designated by (Fazekas 1997)).

Phalaena (*Geometra*) *obscurata* Borkhausen (1794): Natur. eur. Schmett. 5: 537. Emendation of *obscuraria* Villers.

Geometra lividaria Hübner (1799): Samml. Eur. Schmett. 5 Geometrae (1). Syntype(s). Junior synonymy proposed by Prout (1915).

Nychiodes lividaria coloxaria Costantini (1916): Atti Soc. Nat. Modena (5) 3:17 (Italy: Monte Gibbio; Castelvetro; Ligorzano). Syntype(s). Junior synonym of *N. obscuraria* (Fazekas 1997), confirmed by Leraut (2009) and Flamigni et al. (2007), here confirmed.

Nychiodes obscuraria teriolensis Wagner (1927): in Schwingenschuss e Wagner, Z. öst. EntVer 12 (7): 69 (southern Dalmatia; Tyrol). Syntype(s). Junior synonym of *N. obscuraria* (Fazekas 1997), confirmed by Leraut (2009) and Flamigni et al. (2007), here confirmed.

Nychiodes obscuraria ticina Wehrli (1941): in SEITZ, Gross-Schmett. Erde 4 (Suppl.): 437, pl. 37c (Switzerland: Tessin near Biasca). Lectotype ♂, Paralectotype ♀ (ZFMK, designated by Fazekas (1997), externally examined). Junior synonym of *N. obscuraria* (Fazekas 1997), confirmed by Leraut (2009) and Flamigni et al. (2007), here confirmed.

Material examined. Italy: **Puglia:** ♂, Gravina di Laterza (TA), 300 m, 16 vi 1999, leg. Gentile, Palladino, Sciarretta, dissected genitalia 1019 (CAS); **Molise**: ♂, Pietrabbondante (IS), Colle S. Eramo, 1100 m, 24 vii 1996, leg. A. Sciarretta, dissected genitalia 1018 (CAS); ♂, **Piemonte**, Val di Susa Chiomonte LEP-SS-01052 (CREA-FL); **Basilicata**: ♂, Lucania, Monte Caperino, Pietrapertosa, 1.400 m, 27 vii 1974, leg. P. Parenzan, Lep 15073 (ZSM); ♂Lucania, F. Camastra, Cant. Inferno, 550 m, 9 vii 1975 (ZMS); ♀, Pietrapertosa (Potenza), 1000 m, 18 vii 1971 (SNSB-ZSM); **Veneto**: ♂, Garda (SBSN-ZSM); **Trentino**: 13 ♀, 20♂, Suditirol (SNSB-ZSM); **Liguria**: ♀, Pegli (SNSB-ZSM); **Abruzzo**: 3♀, 8♂, Gran Sasso (SNSB-ZSM); ♂, Appennino centrale, Montagna-Grande, 1000 m, 18 vi 1929, leg. F. Dannehl (SNSB-ZSM); ♂, **Lazio**: Monti Sabini (Subiaco), 5 viii, leg. F. Dannehl (SNSB- ZSM); 2♀, 3♂, **France**: **Provence** (SNSB-ZSM); 6♀, 18♂, **Hautes Alpes** (SNSB-ZSM); ♀, **Digne** (SNSB-ZSM); ♂, **Douelle** (SNSB-ZSM); ♂, **Vernet les Bains** (SNSB-ZSM).

Description of external characters and diagnosis. (Fig. 1B) Wingspan 37–49 mm. Wings grey-brown to dark beige, wing tip slightly darker, spots dark grey to black. Forewing antemedial line curved outwards, medial line absent (important diagnostic character of *N. obscuraria* from other European congeners), postmedial line on M1 curved outwards, slightly inwards, ending in the middle of the anterior inner margin. Forewing subterminal area covered with creamy scales, replaced by brown scales towards costa, wavy line very thin, finely toothed, outer margin dark brown to grey. Discal spots blackish, sometimes pale on anterior part. Terminal line of all wings blackish, narrow, strongly wavy, continuous. Basal half of underside of wing light brown, outer/terminal part dark brown. Only the postmedial line visible on the underside (Müller et al. 2019).

Male genitalia. (Fig. 3B) Dorsal part of valva broadly expanded. Only the basal part of the costa of the valva sclerotized, medially slightly humped. Digitiform extension on the apex of valva present, covered with tiny setae. Ampulla superior clubbed, covered with tiny stout setae, ampulla inferior and sacculus process comparatively close to each other, deeply concave between. Sacculus process strongly reduced. Aedeagus larger than in any other species of the *obscuraria* group, horned needle-like, only slightly shorter than the aedeagus, curved in the middle (Müller et al. 2019).

Female genitalia. (Fig. 2B) Genitalia large. Lamella postvaginalis less sclerotized in the middle, more sclerotized laterally, here curved and with curved extension. Ductus bursae membranous, longitudinally striate, of the same length as the membranous corpus bursae. Signum stellate, small (Müller et al. 2019).

Variation. The species shows high variation in size and colour (from light brown to dark forms). The distance between forewing median lines varies in different individuals. Additionally, the degree of light irroration on wings varies (Müller et al. 2019).

Genetic data. BIN BOLD: ACE5020 (n = 7 from France and Italy: regions Trentino-Alto Adige, Piedmont, Emilia-Romagna, Basilicata), BIN-sharing with one of the two Calabrian clusters of *N. ragusaria* (see above) and diverging from the typical cluster of *N. ragusaria* (BOLD: AAE5581) by 1.55% in BOLD barcode gap analysis. The identity of all barcoded specimens was verified by dissection of genitalia.

Discussion

The two species studied exhibit very similar external morphology. However, examination of the genitalia allows for clear discrimination between *N. obscuraria* and *N. ragusaria*. Our results reveal a case of mitonuclear discordance as the conflict between morphology and molecular data demonstrates. In nine of the 24 Calabrian samples examined of *N. ragusaria*, BIN sharing was found with *N. obscuraria*, while the morphology of the genitalia clearly indicated *N. ragusaria*. In addition, we observed a case of exact haplotype sharing between *N. obscuraria* from Basilicata (Lagonegro, Italy) and *N. ragusaria* from the Pollino Massif area, being around 40 km apart. Therefore, in this case, discrimination at a specific level must rely on morphology, which clearly delimits the two species. The P-distance between the two haplotype clusters of *N. ragusaria* from southern Italy (including *N. obscuraria* from Basilicata) is 1.55% in the BOLD DNA barcode gap analysis.

Haplotype sharing as observed for the pair *Nychiodes obscuraria – N. ragusaria* also occurs in other taxa in the Geometridae family. In the allopatric *Eupithecia manniaria* and *E. conterminata*, haplotype sharing was reported between Scandinavian and Alpine populations separated by a very large distance (Scalercio et al. 2021). In the sympatric *Scopula frigidaria* and *S. ternata*, identical haplotypes were found at a distance not lower than 580 km (Hausmann et al. 2013). Given the observed geographic pattern of the pair *Nychiodes obscuraria – N. ragusaria*, we can hypothesize the occurrence of genetic introgression from *N. obscuraria* into *N. ragusaria* as also observed in the species pair *Perizoma hydrata – P. affinitata* (Hausmann et al. 2011). The slight divergences, and - in one case - also identical haplotypes, suggest that introgression has probably taken place several times, possibly also recently. Incomplete lineage sorting is unlikely because of the geographic distribution of the haplotypes. In fact, both haplotypes were found only in a small geographic area close to the potential contact zone of both species. Interestingly, we found that the *obscuraria* haplotype was found only at mountain sites south of the Pollino Massif, suggesting some kind of physiological advantages for mountain populations of *ragusaria*. Based on mitochondrial data this cannot be studied in further detail, but physiological studies and genomics would allow assessing the altitudinal segregation of mitonuclear discordance in much higher detail.

Regarding the geographical distribution, Figs 4, 5 demonstrate that *N. ragusaria* is present from Sicily to the Pollino Massif, while *N. obscuraria* is distributed from Basilicata, Latium, and Molise to northern Italy and France. Further research and more material with additional dissections and DNA barcodes is required to investigate the exact boundaries of the distribution areas of both species, in order to verify or reject a potential sympatric presence of both species in south-western Basilicata, elucidating further the patterns of species circumscription. Additionally, experimental investigation of the potential occurrence of hybridisation is necessary to better understand the discordance between genetics and morphology, which has – so far - only been observed in the Calabrian mountains above 840 m.

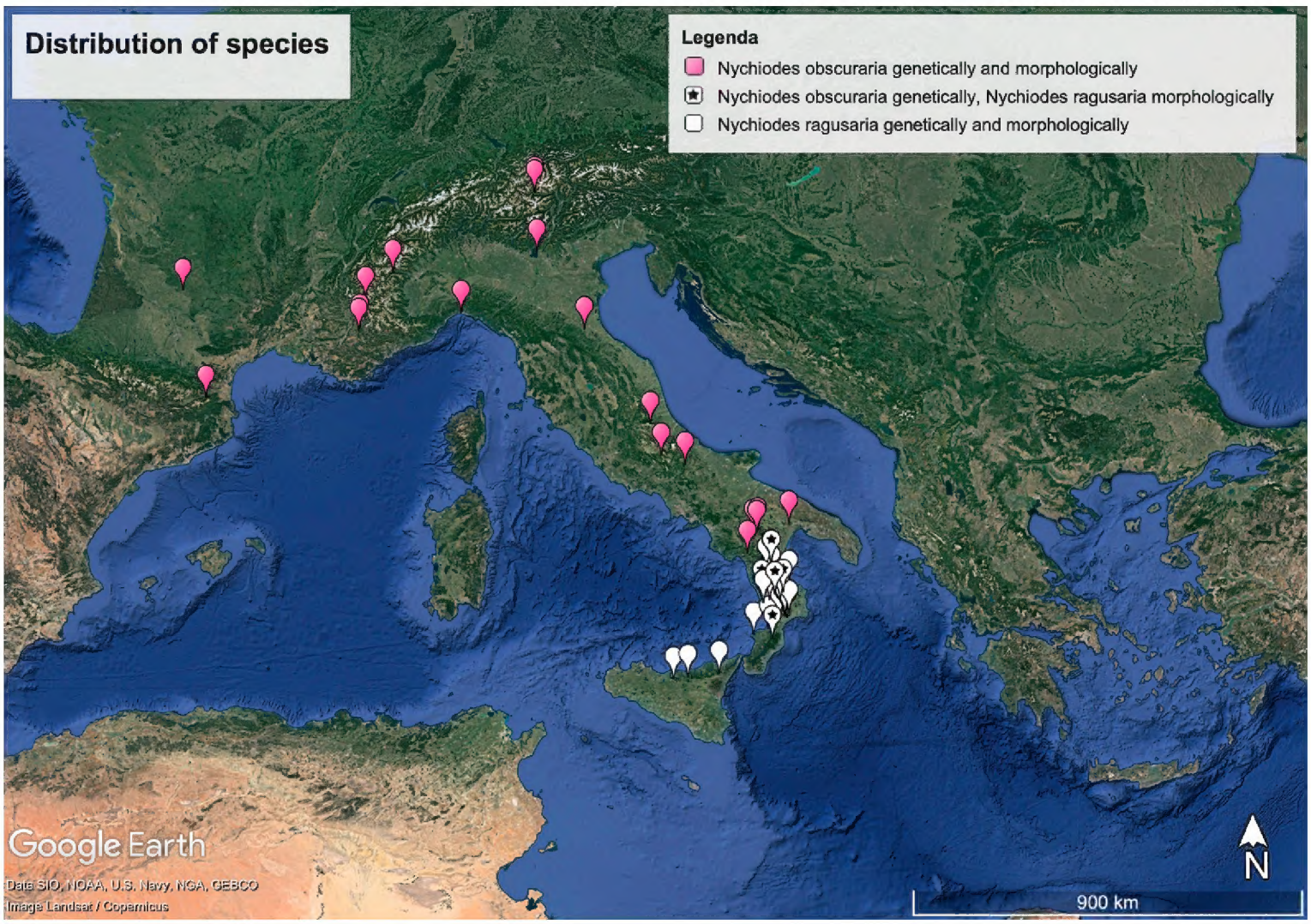

Figure 4. Distribution map of *Nychiodes ragusaria* and *N. obscuraria*. Google Earth Pro (ver. 7.3.6.9345).

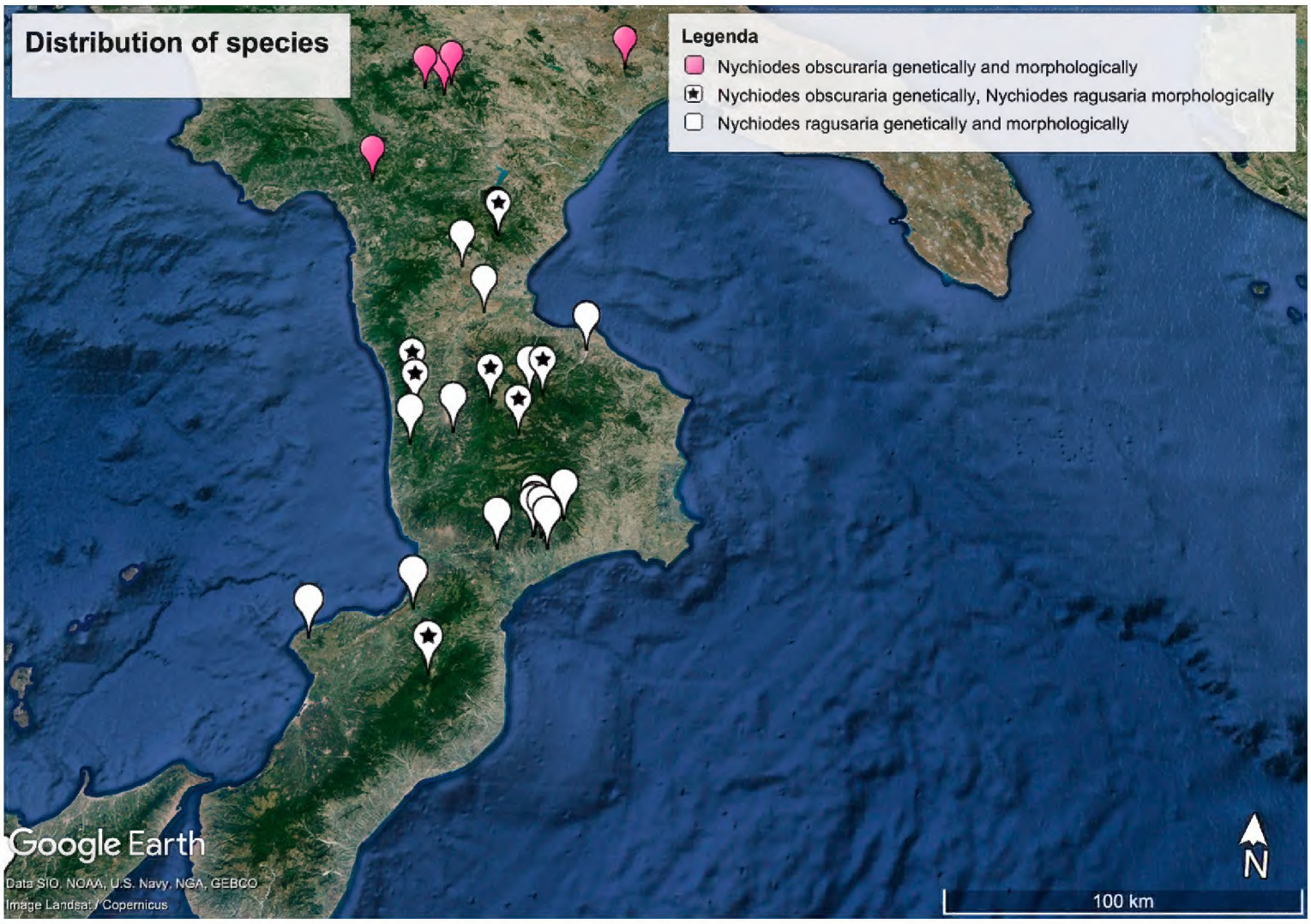

Figure 5. Contact area between *Nychiodes ragusaria* and *N. obscuraria*. Google Earth Pro (ver. 7.3.6.9345).

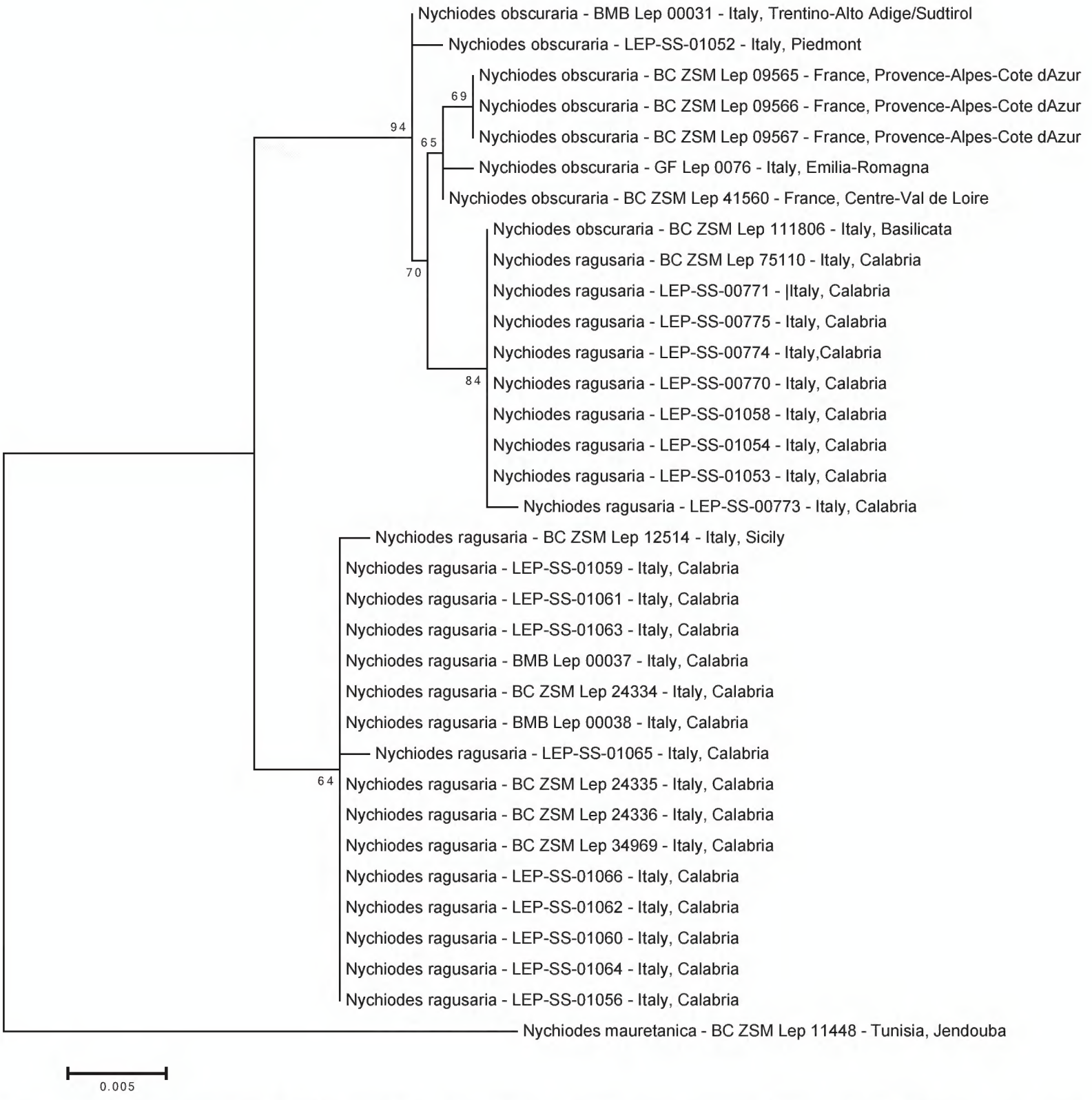
Nychiodes obscuraria - BMB Lep 00031 - Italy, Trentino-Alto Adige/Sudtirol
Nychiodes obscuraria - LEP-SS-01052 - Italy, Piedmont
Nychiodes obscuraria - BC ZSM Lep 09565 - France, Provence-Alpes-Cote dAzur
Nychiodes obscuraria - BC ZSM Lep 09566 - France, Provence-Alpes-Cote dAzur
Nychiodes obscuraria - BC ZSM Lep 09567 - France, Provence-Alpes-Cote dAzur
Nychiodes obscuraria - GF Lep 0076 - Italy, Emilia-Romagna
Nychiodes obscuraria - BC ZSM Lep 41560 - France, Centre-Val de Loire
Nychiodes obscuraria - BC ZSM Lep 111806 - Italy, Basilicata
Nychiodes ragusaria - BC ZSM Lep 75110 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-00771 - |Italy, Calabria
Nychiodes ragusaria - LEP-SS-00775 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-00774 - Italy,Calabria
Nychiodes ragusaria - LEP-SS-00770 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01058 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01054 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01053 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-00773 - Italy, Calabria
Nychiodes ragusaria - BC ZSM Lep 12514 - Italy, Sicily
Nychiodes ragusaria - LEP-SS-01059 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01061 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01063 - Italy, Calabria
Nychiodes ragusaria - BMB Lep 00037 - Italy, Calabria
Nychiodes ragusaria - BC ZSM Lep 24334 - Italy, Calabria
Nychiodes ragusaria - BMB Lep 00038 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01065 - Italy, Calabria
Nychiodes ragusaria - BC ZSM Lep 24335 - Italy, Calabria
Nychiodes ragusaria - BC ZSM Lep 24336 - Italy, Calabria
Nychiodes ragusaria - BC ZSM Lep 34969 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01066 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01062 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01060 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01064 - Italy, Calabria
Nychiodes ragusaria - LEP-SS-01056 - Italy, Calabria
Nychiodes mauretanica - BC ZSM Lep 11448 - Tunisia, Jendouba
94
69
65
70
84
64
0.005

Figure 6. Maximum likelihood tree of 33 French and Italian specimens of the *Nychiodes obscuraria* species-group, with *N. mauretanica* (Tunisia) as outgroup. Built with Mega X, Kimura 2 parameter, 100 replicates, bootstraps indicated on nodes. Scale bar: 0.5%.

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